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Brief Clinical Report

Deletion of Small Nuclear Ribonucleoprotein Polypeptide N (*SNRPN*) in Prader-Willi Syndrome Detected by Fluorescence In Situ Hybridization: Two Sibs With the Typical Phenotype Without a Cytogenetic Deletion in Chromosome 15q

Tatsuya Ishikawa, Tetsuya Kibe, and Yoshiro Wada
Department of Pediatrics, Nagoya City University Medical School, Nagoya, Japan

The small nuclear ribonucleoprotein polypeptide N (*SNRPN*) gene is regarded as one of the candidates for Prader-Willi syndrome (PWS). We describe two sibs with typical PWS presenting deletion of *SNRPN* detected by fluorescence in situ hybridization (FISH). Neither a cytogenetically detectable 15q12 deletion nor a deletion for the *D15S11, D15S10,* and *GABRB3* cosmid probes were found in either patient. This implies a smaller deletion limited to the PWS critical region. FISH with a *SNRPN* probe will permit analysis of PWS patients with limited deletions not detectable with other probes.

KEY WORDS: fluorescence in situ hybridization, chromosome mapping, DNA probes, Prader-Willi syndrome, sibling relations, small nuclear ribonucleoproteins

INTRODUCTION

Prader-Willi syndrome (PWS; McKusick 176270) [1994] is a neuroendocrine disorder characterized by hypotonia, hypomentia, hypogonadism and obesity. In 1981, the segment between 15q11 and q13 was proposed to be the PWS critical region [Ledbetter et al., 1981]. A deletion in chromosome 15 in PWS was shown to be exclusively of paternal origin [Butler and Palmer, 1983], and maternal uniparental disomy was found in nondeletion PWS [Nicholls et al., 1989]. Based on this evidence, gene(s) in the PWS chromosome region have been regarded as being imprinted via epigenetic modification of the active allele.

Fluorescence in situ hybridization (FISH) has been utilized to establish the probe order in the chromosome 15q11–q13 region [Kuwano et al., 1992; Knoll et al., 1993], and it was shown to be superior to high resolution chromosome banding [Delach et al., 1994; Bettio et al., 1995]. In 1992, *SNRPN* was proposed as a candidate for the gene involved in PWS [Özçelik et al., 1992]. Although we had already reported two sibs with typical PWS without a cytogenetic deletion in 15q11–13 [Ishikawa et al., 1987], development of medical genetics led to further evaluation of our patients, and we describe here the deletion of *SNRPN* detected by FISH.

CLINICAL REPORT

The study was carried out on two sibs, patients 1 and 2 (age 22 and 19 years, respectively). They fulfilled the consensus diagnostic criteria for PWS [Holm et al., 1993], having a total score of 10.5. Recent clinical observations were no menarche in either patient, and noninsulin-dependent diabetes mellitus in patient 2.

FISH was conducted according to the protocol recommended by the manufacturer (Oncor). The cosmid probes derived from the *D15S11, SNRPN, D15S10* and *GABRB3* (Oncor Catalog Numbers P5150, 5152, 5153 and 5130, respectively) loci were used for analysis of metaphase spreads. Twenty cells were examined for the presence of fluorescein signals from each cosmid probe in chromosome 15 homologs. A control cosmid containing probe PML cohybridized to identify chromosome 15 (15q22).

For *SNRPN,* either no hybridization signal or a single reduced signal for one chromosome 15 homolog was detected in any of 20 cells from each patient (Fig. 1): no

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Address reprint requests to Dr. Tatsuya Ishikawa, Department of Pediatrics, Nagoya City University Medical School, Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan.

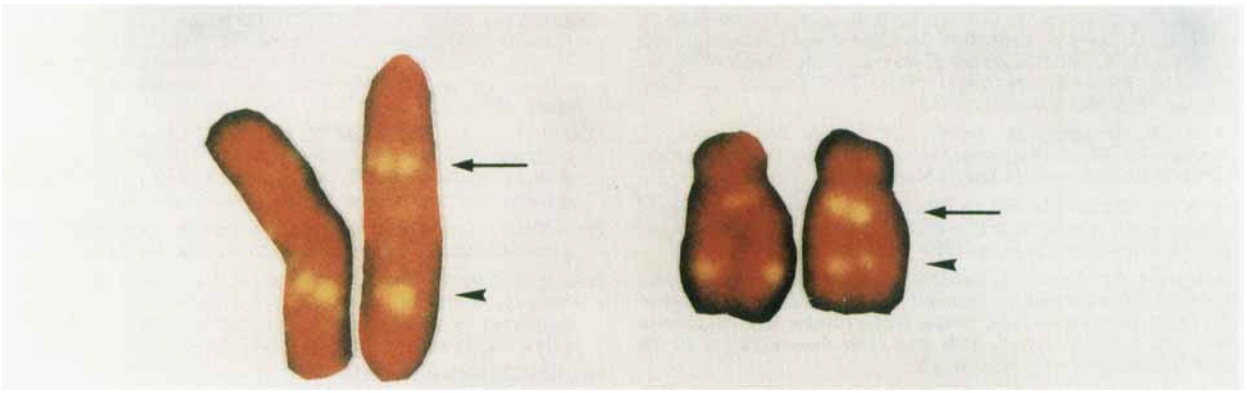

Fig. 1. FISH with cosmid probes *SNRPN* (arrows) and PML (arrowheads), as a control for 15q22, in chromosome 15 homologs from two representative nuclei. **Left** pair: no fluorescein signal of *SNRPN* on one homolog. **Right** pair: a reduced signal of *SNRPN* on one homolog, differing from the bright signal on the other.

signal in three cells and a single reduced intensity in the remaining 17 from patient 1, and no signal in 8 cells and a single reduced intensity in the remaining 12 from patient 2. For *D15S11, D15S10* and *GABRB3,* both chromosome 15 homologs hybridized clearly to the cosmid probes in all 20 cells from each patient.

DISCUSSION

It is now apparent that PWS is caused by the loss of the function of a gene or several genes on the paternal segment, 15q11.2-q12. In most patients, a deletion has been reported in 15q11–q13 and/or the segment from *ZNF127* (*D15S9*) to *D15S12.* Determination of whether or not a nondeletion PWS patient with the typical phenotype has *SNRPN* should be important as to identification of areas of disease loci, since *SNRPN* has been mapped to the minimal critical deletion region for PWS [Özçelik et al., 1992; Mutirangura et al., 1993], and only the paternal *SNRPN* allele was shown to be expressed in humans [Glenn et al., 1993; Reed and Leff, 1994; Nakao et al., 1994]. However, there have been few reports on a pair of PWS sibs, family S [Reis et al., 1994; Buiting et al., 1995] and family O [Sutcliffe et al., 1994; Buiting et al., 1995], besides single PWS patients [Buiting et al., 1994; Sutcliffe et al., 1994], having an identified microdeletion encompassing *SNRPN.*

In our patients, no cells contained both homologs clearly labeled by the cosmid probe derived from the *SNRPN* locus. It is possible that the bright signal of *SNRPN* on one homolog is derived from the maternal allele, which is inactive, while there is no or only a reduced signal on the other from the paternal allele, which reflects localized deletion of the paternal *SNRPN* gene. Although cosmid probes for *D15S13* and *D15S63* were unavailable for this study, no other abnormalities were detected in the 15q11–q13 region, including the *D15S11, D15S10* and *GABRB3* loci. Our patients, therefore, have very localized deletions involving the PWS critical region, which encompasses *SNRPN.*

Furthermore, familial cases of PWS other than monozygotic twins are quite rare [Kennerknecht, 1992; Örstavik et al., 1992; Woodage et al., 1994; Reis et al., 1994]. Our familial cases are likely to be additions to the core patients with the shortest region of deletion in PWS. FISH involving a *SNRPN* probe will be particularly valuable for analyzing PWS patients with limited deletions not detectable with other probes.

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